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ENVIRONMENTAL TEMPERATURE AND THYROID ACTIVITY IN THE LIZARD, *SCELOPORUS OCCIDENTALIS*

K. W. Chiu, W. G. Lynn and J. P. Leichner

Department of Biology, The Catholic University of America, Washington, D. C. 20017

A relation between environmental temperature and the secretory activity of the thyroid gland has been reported for a number of species of lizards on the basis of both field and laboratory studies (see reviews, Lynn, 1960, 1970). It has been suggested that the influence of temperature change upon the thyroid is an indirect one, mediated by the hypophysis (Eakin, Stebbins and Wilhoff, 1959). Increase in temperature is assumed to result in a rise in the release of TSH from the hypophysis and decrease in temperature in a lowering of TSH release. The present expriments were designed to further investigate the relationship between temperature and the hypophyseal-thyroidal axis by a study of radioiodine incorporation by the thyroid in intact, hypophysectomized and sham-hypophysectomized animals maintained at various temperatures ranging from 15° to 38° C and by examining the effects of TSH administration to hypophysectomized animals at the highest temperature, 38° C.

Materials and Methods

A total of 200 specimens of *Sceloporus occidentalis* (Baird and Girard) were purchased from a supply company in California. Before being used for experimentation, the animals were kept for at least one week at room temperature (26 to 33° C) with an 8-hour daily period of illumination. They were fed with larval and adult specimens of *Tenebrio* and water was available to them at all times.

At the end of this period, fully adult lizards of fairly uniform weight were selected and these were divided into groups placed in constant temperature chambers kept at (a) $21 \pm 2°$ C and $30 \pm 1°$ C (experiments performed in October, 1968) and (b) $15 \pm 1°$ C, $30 \pm 1°$ C and $38 \pm 1°$ C (experiments performed in September, 1969). This choice of temperatures was guided by the fact that they cover the range from minimum to maximum voluntary temperatures reported for the species (Brattstrom, 1965; Cunningham, 1966; McGinnis, 1966). After one week in the constant temperature chambers, each group was subdivided into three sets of animals as follows: (A) intact, untreated animals; (B) animals with the pars distalis of the hypophysis removed; (C) sham operated animals. The number of specimens in each group varied and will be indicated in the results (Table I).

At the end of the second week at the designated temperature (*i.e.*, one week after operation), each animal was given an intraperitoneal injection of 5 μc of carrier-free I^{131}. The level of radioactivity in the thyroid region was then measured at 24-hour intervals for a period of seven days.

TABLE I

The percentage uptake of I^{131} by the thyroid gland of the western fence lizard, Sceloporus occidentalis
Mean ± S.E.

Days after I^{131} inj.	Untreated controls				Sham-operated controls				Pars distalis removed			
	38 ± 1°	30 ± 1°	21 ± 2°	15 ± 1°	38 ± 1°	30 ± 1°	21 ± 2°	15 ± 1°	38 ± 1°	30 ± 1°	21 ± 2°	15 ± 1°
	[4]	[9]	[4]	[6]	[5]	[8]	[4]	[4]	[6]	[9]	[8]	[6]
1	16.0 ± 0.5	19.9 ± 3.5	19.1 ± 3.9	2.0 ± 0.4	19.2 ± 1.0	21.2 ± 3.9	25.3 ± 2.5	2.6 ± 0.4	2.4 ± 0.7	8.5 ± 2.1	8.8 ± 1.9	2.8 ± 1.5
2	22.1 ± 7.3	33.1 ± 5.8	35.0 ± 5.5	3.9 ± 0.8	30.8 ± 4.9	33.3 ± 5.4	41.5 ± 3.7	6.0 ± 0.6	3.7 ± 1.4	13.4 ± 3.0	14.2 ± 3.0	7.1 ± 3.5
3	27.1 ± 5.6	41.8 ± 5.4	43.4 ± 8.5	6.2 ± 1.4	34.7 ± 6.7	41.4 ± 5.8	52.8 ± 4.0	8.8 ± 0.5	3.5 ± 1.7	16.7 ± 4.0	19.1 ± 4.4	10.1 ± 4.9
4	30.8 ± 10.2	47.6 ± 5.8	50.5 ± 7.8	7.2 ± 2.0	37.8 ± 9.1	46.8 ± 5.6	59.1 ± 5.3	10.6 ± 0.4	4.1 ± 2.3	19.1 ± 4.4	22.2 ± 4.8	13.1 ± 6.1
5	32.2 ± 12.0	50.7 ± 5.7	55.7 ± 8.3	9.7 ± 2.2	46.6 ± 3.1	49.4 ± 5.5	64.1 ± 5.9	12.7 ± 0.2	4.5 ± 2.4	20.8 ± 4.6	26.7 ± 5.9	15.4 ± 7.1
6	34.2 ± 13.4	52.6 ± 5.6	57.8 ± 7.6	10.7 ± 2.6††	51.4 ± 0.6	51.6 ± 5.8	68.6 ± 5.4	15.1 ± 0.6	5.1 ± 2.7*†	22.0 ± 4.8**	29.9 ± 7.7**	17.4 ± 8.3***
7	35.6 ± 14.9	54.2 ± 5.8	61.9 ± 8.3	12.6 ± 2.9	52.6 ± 2.2	51.7 ± 5.5	69.8 ± 4.1	16.1 ± 1.3	5.4 ± 3.0	22.3 ± 4.8	31.3 ± 7.9	20.4 ± 9.1

Figures in brackets refer to number of animals.
* Animals with pars distalis removed versus sham-operated controls $P < 0.001$.
** Animals with pars distalis removed versus sham-operated controls $P < 0.01$.
*** Animals with pars distalis removed versus sham-operated controls P = not significant.
† Animals with pars distalis removed: 38 ± 1° versus 30 ± 1° $P < 0.02$.
†† Untreated control: 15 ± 1° versus 30 ± 1° $P < 0.001$.

Measurements of radioactivity were made with a scintillation counter consisting of a $1\frac{3}{4} \times 2$-inch NaI crystal, an RCA 6342A photomultiplier tube with a conventional amplifier, and a binary scaler. The crystal and photomultiplier were mounted in a lead cylinder 5.2 mm thick with a collimating slit measuring 4.0 × 120 mm. This was large enough to cover the area of the thyroid when the ventrum of the animal was properly placed over the slit. The animals remained in the constant temperature chambers throughout the week-long counting period during which they were not fed because it had previously been found that these animals show variations in thyroid activity levels during digestion.

To make a statistical comparison of the percentage I^{131} uptake by the thyroid in the various groups, we have used the mean value of the counts ± the standard error of the mean for each group on the sixth day after radioiodine administration ("t" test). This seems justifiable because we have found in previous experiments that the maximum uptake is usually reached by the fifth day and then remains at approximately the same level for about one week.

The results of these experiments indicated the desirability of a further study to test the effects of TSH administration to hypophysectomized animals. Specimens remaining in stock were used for this experiment. They were kept at 38 ± 1° C for one week, removal of the pars distalis was then carried out, and beginning 24 hours after the operation, all animals were given daily injections of 0.025 USP units of TSH per gram body weight in 0.015 ml of 0.7% saline, or just the saline without TSH, for 7 days. Three hours after the last TSH injection, each animal was given 5 μc of carrier-free I^{131} and the radioactivity in the thyroid region was determined at 24-hour intervals for one week as described above. It is difficult to say whether the TSH dosage used is a "physiological" one for lizards since nothing is known of the secretion rate of the hormone in reptiles. It was chosen because our preliminary studies indicated that it resulted in I^{131} uptakes in hypophysectomized animals that were comparable to those found in normal, unoperated lizards.

Surgical removals of the pars distalis and sham-operation were performed under anesthesia with sodium pentabarbital (Nembutal, Abbott). The TSH was purchased from Nutritional Biochemicals Corporation and was reconstituted with 0.7% saline to a concentration of 10 USP units per ml.

Results

General observations

Lizards kept at 15° C became sluggish within a short time and remained inactive throughout the experiment. Those at 21° C were quiescent but, unlike those at 15° C, would move about for short periods when disturbed. At 30° and 38° C the lizards were highly active and very excitable, especially at the latter temperature. Only five specimens of the 73 used in this experiment died during the course of the work. One of these was a sham-operated animal kept at 15° C. The other four were lizards kept at 38° C, two of the four untreated controls and two of the five sham-operated animals.

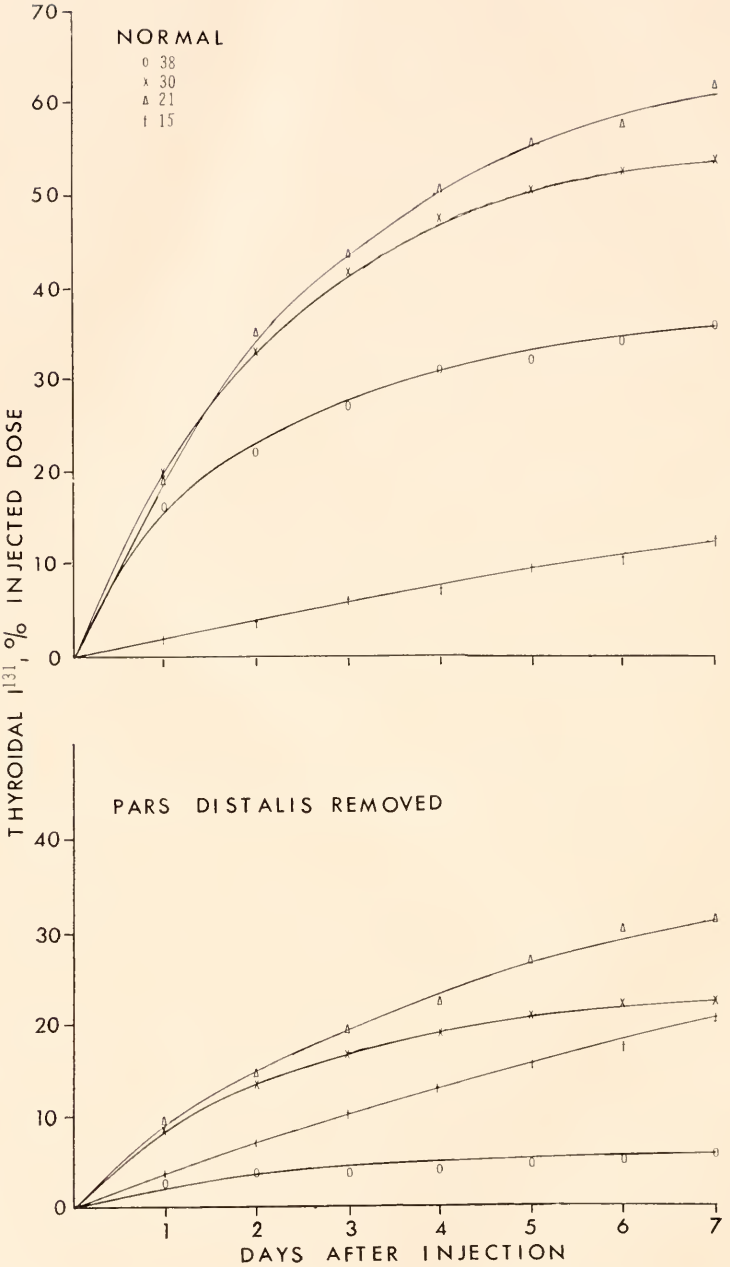

FIGURE 1. Radioiodine uptake by the thyroid, in per cent of injected dose, at various temperatures in unoperated *Sceloporus* and in those with the pars distalis of the hypophysis removed.

I^{131} accumulation by the thyroid

The percentage of I^{131} incorporated by the thyroid gland on successive days after injection is given in Table I for all groups except those given TSH treatment and these data are shown graphically in Figure 1. The data recorded for 30° C represent combined figures for an experiment carried out in two different years (Oct. 1968 and Sept. 1969). Statistical analysis showed no significant difference

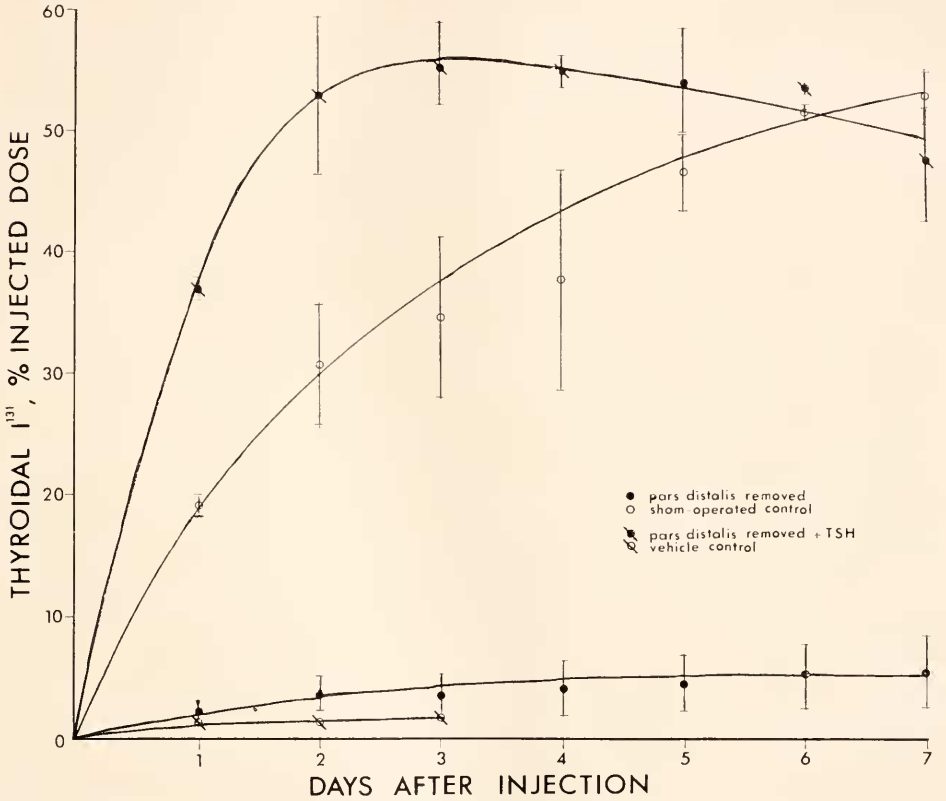

FIGURE 2. The effect of TSH administration upon radioiodine uptake by the thyroid, in per cent of injected dose, in *Sceloporus* with pars distalis removed and in sham-operated animals.

for these two groups of animals and it has seemed justifiable to combine the figures in the interest of brevity. It will be noted that intact, untreated controls kept at any of the high temperatures (21°, 30° or 38° C) showed a gradual rise in radioactivity in the thyroid region during the first 5 days after administration of the isotope and then a levelling off during the sixth and seventh days. However it is noteworthy that the maximum levels reached did not differ significantly at these three temperatures. Although the maximum figure for 38° C appears to be lower than those for 21° C and 30° C, statistical analysis shows that this difference is not significant ($P > 0.8$). At the lowest temperature (15° C), the untreated

controls showed a much lower rate of accumulation and no tendency to level off during the 7-day counting period. This was also true for sham-operated animals and operated animals maintained at this temperature. Comparison of the figures for untreated controls with those for sham-operated animals shows that the sham-operation had no significant effect upon I^{131} uptake by the thyroid. Surgical removal of the pars distalis of the hypophysis resulted in a significant decrease in thyroidal accumulation by the thyroid at all of the high tempertures (21°, 30° and 38° C) but hypophysectomized animals kept at 15° C showed no significant difference in radioiodine uptake as compared with intact or sham-operated animals at that temperature.

The percentage uptake of I^{131} by the thyroids of hypophysectomized lizards injected with TSH is shown graphically in Figure 2. It may be seen that TSH administration resulted in a great increase in radioiodine accumulation especially during the first two days after injection of the isotope, which reached a maximum by the third or fourth day and then showed a slight decline.

Discussion

The present results indicate a general pattern of I^{131} accumulation in the thyroid of *Sceloporus occidentalis* which is similar to that found for other squamate reptiles such as *Gekko* (Chiu *et al.,* 1967) and *Thamnophis* (Chiu and Lynn, unpublished) and for the turtles *Pseudemys* and *Terrapene* (Shellabarger *et al.,* 1956). However, it should be noted that other experiments on *Sceloporus occidentalis* and *Anolis carolinensis* (Kobayashi and Gorbman, 1959) and on *Xantusia henshawi* (Buckingham, 1970) have shown a more rapid rise in thyroidal radioiodine to reach a maximum value by the second day after I^{131} injection with a retention at this value for at least six more days, and still another study on *Anolis carolinensis* (Lynn *et al.,* 1965) indicates that, after reaching maximum levels at about two days, the levels fall rapidly over the next four days, especially in lizards kept at high temperature (35° C).

Comparison of data on radioiodine uptake by the thyroid in intact, untreated animals at the various temperatures used in the present study shows, rather surprisingly, that over the range of 21° C to 38° C, there is no evidence of any significant temperature-related change in thyroid activity. Only at 15° C is the thyroid functioning significantly inhibited. This would seem to indicate that there is a certain critical temperature, somewhere between 15° C and 21° C below which the thyroid gland is relatively inactive but that above this critical temperature the gland reaches a level of activity that is not modified by further temperature increase, at least over the 21° C to 38° C range. It appears that this finding by itself would oppose the theory of a direct effect of temperature change upon the thyroid in the sense of a regular increase in physiological activity with every temperature rise.

Moreover, it is well known that the functioning of the thyroid is under the control of a hypophyseal thyrotropic hormone. This has been demonstrated for the species used in these experiments (Gorbman, 1946; Eakin *et al.,* 1959), as well as for other lizards such as *Anolis* (Nussbaum, 1963; Lynn *et al.,* 1965) and *Xantusia* (Buckingham, 1970). In the present study, as shown by the graphs for the animals with the pars distalis of the hypophysis excised, absence of the hypo-

physeal hormones results in a sharp decrease in thyroid activity in all animals except those kept at 15° C. This supports all previous studies on the importance of TSH in normal thyroid functioning in lizards, and also indicates that at 15° C the thyroids of intact, untreated animals are functioning at no higher level than are those of animals that completely lack TSH.

Wilhoft (1958) and Eakin *et al.* (1959) found that fence lizards kept at 35° C are much more active than those kept at room temperature and advanced the hypothesis that this is related to increased thyroid activity at higher temperature which is, in turn, due to a heightened release of TSH from the hypophysis. In view of the present results, it seems more likely that the role of temperature in thyroid activation is simply a permissive one. Below a certain temperature level there is no response to temperature change; above that level the thyroid is active regardless of the precise temperature, at least over the 21° to 38° C range. This is supported also by work done in this laboratory on *Phrynosoma* (Leichner, unpublished). This would mean either that TSH release from the hypophysis is subject to an "all or none" relation to temperature or, alternatively, that the responsiveness of the thyroid to TSH stimulation is so related.

Despite the small number of animals available, it seems clear that the dosage of TSH used (0.025 USP units per gram body weight daily for seven days) was sufficient to increase the uptake of radioiodine by the thyroids of hypophysectomized specimens to approximately the normal level for intact animals maintained at the same temperature (38° C). However, it must be emphasized that, although the level of uptake reaches similar values in these groups, the precise pattern of radioiodine accumulation in the hypophysectomized animals given exogeneous TSH at a single dosage level is quite different from the normal pattern. In the latter the accumulation is gradual over the seven-day counting period, whereas in the former the highest level is reached by the third day and then declines. This raises the interesting possibility that there is normally a continuous and constant rate of release of TSH from the hypophysis in many squamate and turtle species which accounts for the pattern of thyroidal accumulation of I^{131} in *Gekko*, *Thamnophis*, *Terrapene* and *Pseudemys*, and *Sceloporus*. When a cyclic fluctuation in endogeneous TSH production and release occurs, a different pattern of thyroidal I^{131} accumulation comparable to those reported for *Anolis* (Lynn *et al.*, 1965) and *Xantusia* (Buckingham, 1970) would result. In the present study, the pattern found for hypophysectomized *Sceloporus* receiving TSH injections would also be explicable on this basis.

Summary

The relationship between temperature and the hypophyseal-thyroidal axis in *Sceloporus occidentalis* has been investigated by a study of radioiodine incorporation by the thyroid in intact animals, and animals with the pars distalis removed, kept at temperatures of 15°, 21°, 30° and 38° C. Over the range of 21 to 38° C there was no evidence of any significant temperature-related change in the normal and experimental animals, although the thyroid activity was reduced after the operation. At 15° C the thyroid functioning was significantly inhibited and there was no difference between the normal and experimental animals. It is suggested that the role of temperature in thyroid activation is a permissive one. Data for

I^{131} incorporation in hypophysectomized animals receiving TSH therapy offers a possible explanation for the different patterns of thyroidal I^{131} accumulation observed in different forms of lizards.

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